Cell

Building Bridges for Spinal Cord Repair

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For nearly a century, neuroscientists have sought to restore neurological function across spinal cord lesions. Lu et al. now present significant progress toward this goal, showing in rats that transplanted neural stem cells establish a functional bridge across completely transected spinal cords.

Spinal cord injury (SCI) in adult mammals causes large zones of necrosis to develop at the site of injury, creating gaps or voids that prevent communication between brain and spinal cord. Axons cannot cross this gap; any attempt at regeneration is met with failure as axons either retract from the injury site or linger near the lesion borders. Scientists have long believed that intraspinal transplants can be used as bridges or functional relays capable of restoring communication across the injury site. Successful graft integration and subsequent growth of axons into or out of the transplant depends on several factors, including lesion size, time to transplantation (acute versus chronic), source of transplant (that is, neurons, Schwann cells, olfactory ensheathing glia, or bone marrow stromal cells), developmental stage of transplanted cells, presence of supportive matrices, use of adjunct therapies (such as growth factors or immunosuppressants), genetic matching, and manipulation of the glial scar (for example, by treatment with chondroitinase) (Reier, 2004). Until now, such approaches have met with limited success.

In this issue of *Cell*, Lu et al. (2012b) show in rats that it is possible to dramatically improve the survival, growth, and integration of neural transplants and to functionally bridge a complete spinal resection lesion (2 mm gap), a formidable preclinical SCI injury model, by using suspensions of embryonic rat or human neural stem cells (NSCs). In all cases, transplanted cells are engineered to express enhanced green fluorescent protein (EGFP), allowing unprecedented visualization of cellular growth and connectivity. Their data show that thousands of graft-derived axons grow effortlessly into host white and gray matter at rates comparable to regenerating peripheral axons (~1–2 mm/day) and can promote recovery of hindlimb function. Notably, these effects are achieved without creating neurotrophic gradients or dissolving the glial scar.

Several laboratories have documented anatomical and functional repair in the injured spinal cord by using various transplantation techniques (Sahni and Kessler, 2010); however, the current report documents what is, to date, the most robust example of transplant-mediated repair in the central nervous system. It is likely that the success of Lu et al. (2012b) can be traced to techniques used to suspend and deliver cells to the injured spinal cord. Specifically, NSCs are bathed in a cocktail of proliferative and neurotrophic factors mixed into a fibrin gel (Figure 1). When injected into the injury site, this "perfect storm" of proteins and matrix provide sustained trophic support and a protective niche from which thousands of graft-derived axons projected up to 25 mm between the cervical or lumbar spinal cord. Moreover, the transplants were electrically active, acting as relay networks capable of restoring partial function in paralyzed hindlimbs of rats.

Other functional modalities have not been tested, but it is logical to question the broader neurological impact of these transplants. For example, graft-derived choline acetyltransferase (ChAT)-positive axons extend into ventral roots adjacent to the transplantation site. If these axons form functional neuromuscular junctions with skeletal muscle, a difficult task and one that likely requires concomitant trophic cues in the periphery (Deshpande et al., 2006), the newly established segmental circuitry might be useful for controlling axial musculature, sphincter control, or improving breathing. It also would be useful to know whether the transplants can alleviate spasticity, neuropathic pain, or autonomic dysreflexia—that is, aberrant functional changes caused by segmental plasticity below the level of injury. Of course, robust growth and new circuit formation could have the opposite effect of exacerbating pain, spasticity, or autonomic dysreflexia (Brown and Weaver, 2012). Lu et al. (2012a) recently reported that promoting robust axonal regeneration beyond the site of SCI can enhance spasticity that subverts motor recovery.

In the current report, the authors consider possible molecular mechanisms underlying the robust growth of transplanted NSC axons. In a recent landmark paper, the tumor suppressor gene *Pten* was identified as a negative regulator of mTOR-dependent axon growth in the adult spinal cord (Liu et al., 2010). Here, Lu et al. (2012b) show that axonal outgrowth was reduced by ~50% in rats injected with the mTOR inhibitor rapamycin, suggesting that early-stage neurons use mTOR-dependent mechanisms to overcome barriers to axon growth after SCI. However, thousands of axons still extended several millimeters beyond the site of transplantation in rats injected with rapamycin, suggesting that mTOR-independent mechanisms also contribute to this remarkable growth.

Confirming previous data (Josephson et al., 2002), Lu et al. (2012b) find that transplanted embryonic NSCs express mRNA encoding receptors for Nogo,

a myelin inhibitory protein that causes axonal growth cone collapse. From these data, they conclude that insensitivity to myelin-based inhibitors cannot account for the robust axon growth that they observe. Taken alone, their data suggest that there is hierarchical control of mammalian CNS axon regeneration, with neuron-intrinsic regulation trumping the effects of extrinsic factors. However, Itzhak Fischer's group recently discovered that NSCs express lower levels of the receptors protein tyrosine phosphatase sigma (PTPσ) and leukocyte common antigen-related phosphatase (LAR), rendering NSCs less sensitive to the growth inhibitory effects of chondroitin sulfate proteoglycans (CSPGs) (Ketschek et al., 2012). The same group also showed that glial-restricted precursors (derived from embryonic spinal cord cells of similar age to those used in the present study) secrete unknown factors that augment axonal outgrowth across CSPG gradients (Ketschek et al., 2012). Whether NPC-derived glia, which account for ~45% of differentiated cells in the NSC transplants of Lu et al. (2012b), are integral in promoting axonal outgrowth is not determined in the present study.

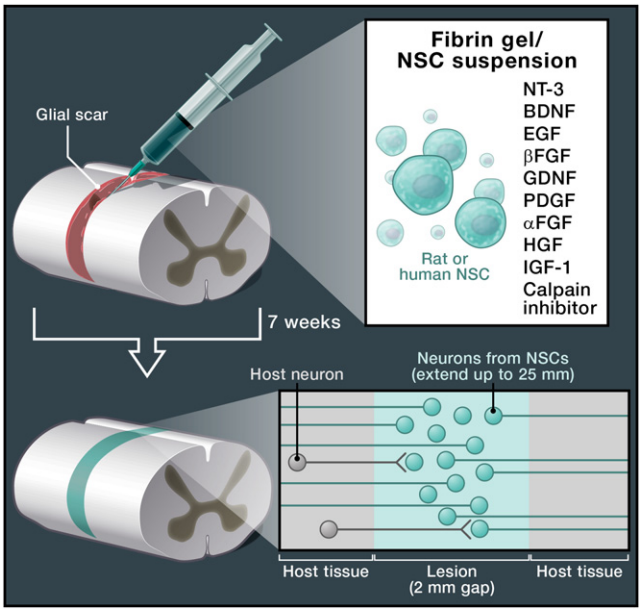

Figure 1. Neural Stem Cell Transplantation for the Repair of Spinal Cord Injury

The schematic illustrates the approach used by Lu et al. (2012b) for promoting repair following spinal cord injury. At 14 days postinjury, a cocktail of growth factors (listed in the pop-out of the gel/cell suspension), a calpain inhibitor, and rat or human embryonic spinal cord neural stem cells (NSCs) are suspended in a fibrin gel, which is then injected into rats with a complete spinal transection (with resection) lesion, filling a gap of ~2 mm. After 7 weeks, a subset of grafted NSCs (green) differentiated into neurons (~30% of grafted cells), which projected axons over multiple spinal segments, bridging cervical and lumbar spinal cord. Host-derived axons (gray) grow into the transplant and form synapses. Only supraspinal (reticulospinal) host axons were traced. NT-3, neurotrophin-3; BDNF, brain-derived neurotrophic factor; EGF, epidermal growth factor; GDNF, glial-cell-line-derived neurotrophic factor; bFGF, basic fibroblast growth factor; aFGF, acidic fibroblast growth factor; PDGF-AA, platelet-derived growth factor; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1.

The magnitude of axonal growth and functional recovery achieved in this report is truly unprecedented, and there is little doubt that these data will prompt excitement about the clinical potential of treating SCI with NSC transplants. However, from both a biological and translational perspective, it will be important to first test whether similar benefits can be achieved in models of spinal contusion injury. Unlike the resection model used here, axons are spared at the site of most human spinal injuries, and residual neurological function is not uncommon. This reality will prompt the inevitable debate of whether transplantation, an invasive procedure, will do more harm than good. While that debate rages on, we can and should revel in the fact that these new data show that it is possible to rebuild the spinal cord or at least partially repair it.

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